METABOLIC PROFILE OF 1,5-DICAFFEOYLQUINIC ACID IN RATS, AN IN VIVO AND IN VITRO STUDY

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ABSTRACT:

To explore the metabolism of 1,5-dicaffeoylquinic acid (1,5-DCQA) in rats, liquid chromatography-mass spectrometry in parallel to diode-array detection was used for the rapid detection/characterization of the metabolites formed in bile, urine, and plasma of rats following oral administration of 1,5-DCQA (160 mg/kg). The methylation and glucuronidation of 1,5-DCQA occurring in vitro using rat liver and small intestinal microsomes and cytosols were studied in comparison with those occurring in vivo, and the enzymes involved were also determined. In addition, the anti-HIV (human immunodeficiency virus) activity of three important metabolites was preliminarily evaluated in MT-4 cells infected with HIV-1. A total of 22 metabolites in vivo and in vitro were identified, including four isomeric O-mono-methylated metabolites (M8–M11), nine isomeric O-di-methylated metabolites (M3, M6, M22, and M12–M17), four isomeric O-mono-methyl-glucuronidated metabolites (M2 and M19–M21), four isomeric O-di-methyl-glucuronidated metabolites (M1, M4, M5, and M7), and one glucuronidated metabolite (M18). The O-methylation positions of three important metabolites (M8, M9, and M12) were determined (3′-, 3′-, and 3′,3″-) by comparing with synthesized standards. The efficacy experiments showed that M6, M9, and M12 could inhibit HIV replication with IC₅₀ values of about 25, 25, and 46 µM, respectively. These results suggest that O-methylation and glucuronidation are two important metabolic pathways of 1,5-DCQA, that both rat liver and small intestine can catalyze such reactions by catechol-O-methyltransferase and UDP-glucuronosyltransferase, and that the HIV-1 inhibitory activity of M8, M9, and M12 is comparable to or slightly weaker than that of 1,5-DCQA.

Dicaffeoylquinic acids (DCQAs) are a class of natural polyphenolic compounds widely distributed in plants, such as fennel (Parejo et al., 2003), mugwort flowering tops (Fraisse et al., 2003), coffee (Moreira et al., 2002), and Eleutherococcus senticosus (Tolonen et al., 2002). Structurally, they are characterized by two caffeic acid molecules connected to one quinic acid molecule through ester bonds. In the past 10 years, DCQAs have been established as an important class of compounds with their potential effects of inhibiting HIV-1 integrase selectively and preventing HIV-1 replication in tissue culture at nontoxic concentrations (McDougall et al., 1998; Robinson et al., 1996a, b; King et al., 1999). HIV-1 integrase is an essential enzyme that mediates integration of the HIV genome into the host chromosomes. Zhu et al. (1999) reported that such inhibition of DCQAs on the HIV-1 integrase is irreversible toward its conserved amino acid residues in the central core domain during catalysis. This work was supported by Grant 30371669 from the National Natural Science Foundation of China and by Grant 2003AA2Z347B from the National High Technology Research and Development Program of China (863 Program).

HIV integrase inhibitors with already existing inhibitors for HIV reverse transcriptase and protease have been suggested to be strongly synergistic (Robinson, 1998). Therefore, DCQAs have drawn more and more attention in the development of the therapy of HIV infection.

However, little is known regarding the metabolism and pharmacokinetics of DCQAs, except for one paper (Takenaka et al., 2000), which reported that no parent compound could be detected in plasma when 3,5-DCQA (3,5-dicaffeoylquinic acid) was orally administered to rats and that 90% of it disappeared from plasma within 30 min after intravenous injection. Recently, we studied the pharmacokinetic behavior of 1,5-DCQA (Fig. 1), which is also a potent and nontoxic inhibitor of HIV-1 replication, like other DCQAs. We observed that 1,5-DCQA did reach the circulation system of rats following oral administration, but its bioavailability was poor (unpublished results). In general, the low bioavailability could be due to poor transport and/or extensive metabolism. During the quantification study of 1,5-DCQA in biological matrices, we found large numbers of unknown compounds eluted together with the parent compound on HPLC, suggesting that metabolism might be one factor leading to its low bioavailability. To address this possibility, we focused our attention on the metabolism of 1,5-DCQA. In the present study, efforts have been made to 1) identify the metabolic pathways of 1,5-DCQA using HPLC, diode-array detection; MS/MS, tandem mass spectrometry; COMT, catechol-O-methyltransferase; UDPGA, uridine 5′-diphosphoglucuronic acid; UGT, UDP-glucuronosyltransferase.

ABBREVIATIONS: DCQA, dicaffeoylquinic acid; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; DAD, diode-array detection; MS/MS, tandem mass spectrometry; COMT, catechol-O-methyltransferase; UDPGA, uridine 5′-diphosphoglucuronic acid; UGT, UDP-glucuronosyltransferase.

an in vivo and in vitro combined method; 2) characterize the structures of its metabolites observed; 3) determine the enzymes involved in the metabolism, and 4) preliminarily evaluate the anti-HIV activity of the important metabolites.

Materials and Methods

Chemicals. Compound 1,5-DCQA and the synthesized standards of M8, M9, and M12 were provided by Beijing Institute of Radiation Medicine (Beijing, China) and authenticated at the National Center of Biomedical Analysis (Beijing, China). β-Glucuronidase from Helix pomatia (type H-1), 3′-adenosyl-L-methionine, uridine 5′-diphosphoglucuronic acid (UDPGA), alamethicin, 3,5-dinitrocatechol, EDTA, dithiothreitol, and Tris HCl were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile was HPLC grade.

HPLC pump equipped with a gradient controller, a Thermo Electron Surveyor diode-array detector, and a Thermo Electron Surveyor Navigator software (version 1.2, Thermo Electron).

LC-MS Analysis. Ion trap-based LC-MS was performed on a Finnigan LC-MS Analysis. Ion transfer capillary temperature of 250°C, spray voltage of −4.20 kV, sheath gas pressure and auxiliary gas pressure of 50 arbitrary units and 15 arbitrary units, respectively, capillary voltage of −24 V, and tube lens offset of −60 V. The mass spectrometer was operated in negative ion mode with a scan range from m/z 150 to 800 (scan rate 0.5 scan/s). Data were collected and analyzed by the Navigator software (version 1.2, Thermo Electron).

HPLC separation was achieved by an Agilent ZORBAX-C18 column (3.0 × 100 mm, 3.5 μm, compounded with an Agilent C18 guard column) (Agilent Technologies, Palo Alto, CA) eluted with a gradient from 5% solvent A (CH3CN) to 75% solvent B (ammonium acetate buffer, 5 mM, pH 5.0) in 30 min and held until 42 min, flow rate 0.4 ml/min. The scan range of DAD was from 200 to 450 nm (rate 0.5 scan/s).

Extraction of Metabolites from Biological Samples. Plasma (800 μl) or other biological samples (200 μl) and 7.2 N HCl (32 μl for plasma and 8 μl for other samples) were added into a test tube, followed by vortexing for 20 s. The solution was then extracted twice with ethyl acetate (2.4 ml for plasma and 600 μl for other samples) by vortexing vigorously for 2 min. After centrifugation at 2000g for 10 min at 4°C, the supernatant was transferred into a conical test tube and evaporated to dryness under a nitrogen stream at 25°C. The residue was reconstituted with 300 μl of the starting HPLC mobile phase, and an aliquot (20 μl) was then injected into the LC-MS system.

Animals and Sampling Procedure. Male Wistar rats (n = 13, 0.20 ± 0.01 kg) were provided by the Institute of Jingfeng Medical Animal Center (Beijing, China). Prior to the experiment the rats were housed, one per metabolic cage, in a temperature-controlled room (22°C) and maintained in a reversed 12-h light/dark cycle with free access to food. After 2 weeks of adaptation to the environment, the rats were randomly divided into three groups for the metabolic studies of 1,5-DCQA in vivo and in vitro.

Biliary Studies. Rats (n = 5) were anesthetized by sodium pentobarbital (30 mg/kg), and a polyethylene cannula was inserted into the bile duct. After the surgery, 1,5-DCQA was orally administered at a dose of 160 mg/kg and bile samples were collected from 0 to 2, 2 to 4, 4 to 6, 6 to 8, and 8 to 10 h after dosing. Sample aliquots collected from each subject were pooled and then stored at −20°C. Before analysis, a 1.0-ml portion of the bile sample (1:1, v/v, in 0.1 M ammonium acetate buffer, pH = 5.0) was acidified to pH = 5.0 with 15 μl of 0.6 M acetic acid and incubated at 37°C for 1 h with or without 1000 U of β-glucuronidase. After cooling, 10 μl of 7.2 N HCl was added, and each sample was centrifuged at 10000g for 10 min. The samples were then treated as mentioned above and analyzed for the identities of the metabolites and their susceptibility to enzymatic cleavage by LC-MS analysis.

Urineary and Plasma Studies. Prior to single oral administration of 160 mg/kg 1,5-DCQA, the rats (n = 4) were fasted overnight with water ad libitum. Urine samples were collected between 0 and 12 and 12 and 24 h postadministration. Sample aliquots collected from each subject were pooled and stored at −20°C until analysis. During the period of collecting urine samples, a volume of 300 μl of blood was sampled in heparinized tubes at 0, 1, 1.5, and 2 h after dosing, followed by centrifugation at 2000g for 10 min to prepare the plasma. Sample aliquots collected from each time point were pooled and then analyzed directly.
Metabolic Studies in Vitro. The preparations of liver and small intestinal cell-free extracts were conducted using the method of Crespy et al. (1999) with some modifications. Briefly, liver or small intestinal mucosa samples from rats (n = 4) were homogenized in ice-cold solvent A (50 mM Tris-HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA, and 1 mM dithiothreitol), followed by differential ultracentrifugation to obtain the 100,000g supernatant as cytosolic fraction and the pellet as microsomal fraction. The microsomes were then resuspended in solvent A containing 10% glycerol. The final solutions were stored at −70°C until use. Cytosolic and microsomal protein concentrations were determined using the Pierce bicinchoninic acid (BCA) protein reagent kit (Pierce Chemical, Rockford, IL).

1,5-DCQA (30 µM) was preincubated with an aliquot of the liver cytosolic fraction (0.05 mg of protein/ml), 5 mM MgCl₂, alamethicin (50 µg/mg protein), and an aliquot of the liver microsomal fraction (0.25 mg of protein/ml) in 0.1 M sodium phosphate buffer (pH 7.4) for 4 min at 37°C. To initiate the reaction, UDPGA (4 mM) and 5-adenosyl-l-methionine (0.32 mM) were added simultaneously to give a final volume of 300 µl. The reaction mixture was incubated at 37°C for 30 min with no agitation after initial mixing, and the reaction was quenched by addition of ice-cold 7.2–N HCl (20 µl). Then, the mixture was treated and analyzed for the identification of the metabolites. In the case of the small intestinal mucosa, the protein concentrations of cytosolic and microsomal fractions were 0.4 and 0.375 mg/ml, respectively. In another experiment to determine the enzyme responsible for the methylation of 1,5-DCQA, 3,5-dinitrocatechol (2.0 mM) was used as a reference.

Results

Metabolic Studies of 1,5-DCQA in Vivo and in Vitro. By comparison with the blank samples, there were many components observed in bile, urine, and plasma of rats following oral administration, which had the typical DAD fingerprints (318–326 nm, 240–252 nm, and 290–300 nm) similar to that of 1,5-DCQA, suggesting that they were the potential metabolites of 1,5-DCQA. Their structures were elucidated by a combination analysis of the mass spectrometry and MS/MS spectra, chromatographic behavior, and response to β-glucuronidase treatment, as well as mass and chromatographic spectral comparison to several synthesized reference substances. There were 14 metabolites in bile (Fig. 2A), 11 metabolites in urine (Fig. 2B), and 8 metabolites in plasma (Fig. 2C) identified.

Incubation of 1,5-DCQA with cell-free extracts of the small intestinal mucosa in low protein concentrations for 30 min led to the disappearance of more than 50% of 1,5-DCQA and the formation of 14 metabolites (Figs. 2D and 3A), including 8 that were identical to those observed in vivo (M2, M4, M5, M8, M9, and M12–M14) and an additional 6 (M10, M11, and M18–M21). In the case of the liver, the same results were obtained (data not shown).

Identification of Metabolites Found in Vivo and in Vitro. A total of 22 metabolites were found in vivo and in vitro. The main characteristic mass fragment ions of 1,5-DCQA and the metabolites were summarized in Table 1.

Parent Drug (M0). M0 was found in urine, plasma, and in vitro incubated samples. Single-stage full-scan mass spectrum of M0 showed a quasi-molecular ion ([M − H]−) at m/z 515, and its MS/MS spectrum showed a number of characteristic fragment ions at m/z 353, 335, 191, and 179 (Fig. 1). M0 was identified as 1,5-DCQA and confirmed by a comparison of its retention time and mass spectrum with reference substance.

Metabolites M3, M6, M22, and M12–M17. M12, M13, and M14 were present in bile, urine, plasma, and in vitro incubated samples, M15, M16, and M17 were found in bile and urine, whereas M3 and M6 were detected only in bile. Incubation of bile with β-glucuronidase could result in the formation of M22 (Fig. 2A). All of them showed the same quasi-molecular ions at m/z 543 ([M − H]−) as well as identical MS/MS fragment ions (full scan) at m/z 367, 349 ([367 − H₂O]−), and 193. Their quasi-molecular ions were 28 Da higher than that of 1,5-DCQA, suggesting that they were the di-methylated metabolites of 1,5-DCQA. Their three MS/MS fragment ions were all 14 Da higher than those of the parent compound at m/z 353, 335, and 179, respectively, suggesting that the two methyl groups were on the two respective caffeoyl groups of 1,5-DCQA. M12 was confirmed as 1,5-O-diferuoylquinic acid by a comparison of its retention time and mass spectrum with reference substance.

Metabolites M1, M4, M5, and M7. M4 and M5 were present in bile, urine, plasma, and in vitro incubated samples, whereas M1 and M7 were found only in bile and urine. They all showed the same quasi-molecular ions at m/z 719 ([M − H]−) as well as identical MS/MS fragment ions (full scan), indicating that they were isomers. Their characteristic MS/MS spectra gave fragment ions at m/z 543 ([M − H − 176]−), and the MS/MS spectrum of M1 still gave another ion at m/z 367. Incubation of bile with β-glucuronidase resulted in their disappearance or decrease and the corresponding appearance (such as M22; Fig. 2A) or increase of the di-methylated metabolites. They were thus identified as the di-methyl-β-glucuronide conjugates of 1,5-DCQA.

Metabolites M8–M11. M8 and M9 were detected in plasma and in vitro incubated samples, whereas M10 and M11 were observed only in incubated samples. They all showed the same quasi-molecular ions at m/z 529 ([M − H]−) as well as identical characteristic MS/MS fragment ions at m/z 353, 367, 179, and 191, indicating that they were the mono-methylated metabolites of 1,5-DCQA. M8 and M9 were confirmed as 1-caffeoyl-5-feruoylquinic acid and 1-feruoyl-5-cafeoylquinic acid, respectively, by a comparison of their retention times and mass spectra with reference substances.

Metabolites M2 and M19–M21. M2 was present in bile, urine, plasma, and in vitro incubated samples, whereas M19, M20, and M21 were detected only in incubated samples. Incubation of bile with β-glucuronidase resulted in the disappearance of M2. They all had the same quasi-molecular ions at m/z 705 as well as identical characteristic MS/MS fragment ions at m/z 353, 367, 179, and 191, indicating that they were the mono-methylated metabolites of 1,5-DCQA. M8 and M9 were confirmed as 1-caffeoyl-5-feruoylquinic acid and 1-feruoyl-5-cafeoylquinic acid, respectively, by a comparison of their retention times and mass spectra with reference substances.

Metabolite M18. M18 was found only in in vitro incubated samples. It showed a quasi-molecular ion at m/z 691 as well as characteristic MS/MS fragment ions at m/z 515, 353, and 529 (a loss of one caffeoyl unit), indicating that it was the glucuronidated metabolite of 1,5-DCQA.

Identification of the Enzymes. In the in vitro studies, 1,5-DCQA and its methylated metabolites could be glucuronidated in the presence of UDPGA, indicating that the enzyme involved in the reactions was UGT; the methylation of catechin-containing 1,5-DCQA and its glucuroconjugate (M18) could be inhibited by 3,5-dinitrocatechol (Fig. 3), a central COMT inhibitor (Singh et al., 2003), confirming that the enzyme involved was COMT.

Anti-HIV Activity of M8, M9, and M12. Both M8 and M9 inhibited HIV replication in MT-4 cells with IC₅₀ (50% inhibitory concentration) of about 25 µM, identical to that of 1,5-DCQA. The IC₅₀ value of M12 was about 46 µM. The results suggested that the inhibitory activity of M8 and M9 was comparable to that of 1,5-DCQA. Although the inhibitory ability of M12 was slightly weaker.
than that of 1,5-DCQA, it was still potent as a HIV inhibitor. The efficacy studies will be detailed elsewhere later.

**Discussion**

1,5-DCQA is a potent and nontoxic inhibitor of HIV-1 integrase. Although the detection of intact 1,5-DCQA in rat plasma revealed that it could be absorbed in its native form, its oral bioavailability was low. In the pharmacokinetic study, large numbers of unknown compounds were found in the matrices of rats in addition to 1,5-DCQA itself, suggesting that the extensive metabolism might be one factor leading to the low bioavailability of 1,5-DCQA. To explore the metabolism of 1,5-DCQA, an in vivo and in vitro combined method was used. The results showed that methylation and glucuronidation were two important metabolic pathways of 1,5-DCQA in rats, and that both rat liver and small intestine could efficiently catalyze such reactions by COMT and UGTs. Thus, the small intestinal mucosa might play a role in the first-pass metabolism of 1,5-DCQA, and the liver would further reduce the level of 1,5-DCQA appearing in the systemic circulation. As a result, large numbers of metabolites were excreted in rat bile and urine. This suggests, at least in part, that metabolism is a limiting step in the bioavailability of 1,5-DCQA. Further work is under way in our laboratory to quantify the amounts of these metabolites by tritium-labeled 1,5-DCQA.

Although King et al. (1999) reported that the biscatechol moieties of DCQAs were absolutely required for the inhibition of integrase, it seemed that mono- or di-methylation occurring on the phenolic hydroxyl(s) of 1,5-DCQA did not significantly alter its anti-HIV activity, because the efficacy studies in vitro revealed that three methylated metabolites, M8, M9, and M12, were also potent in inhibiting the replication of HIV-1. Accordingly, we proposed that other methylated metabolites should also possess the potency, because they had similar structures. If that was the case, part of the anti-HIV effect of 1,5-DCQA in vivo should be due to its methylated products occurring in
the circulation, such as M8, M9, and M12–M14 (Fig. 2C). Thus, the appearance of these circulating bioactive metabolites would explain to some extent why 1,5-DCQA could exert its pharmacological effect even in a low circulation concentration.

Although large numbers of methylated and glucuronidated metabolites in vivo were identified, we could not know the metabolic pathways of 1,5-DCQA clearly until the metabolites observed in vitro were identified. The observations that two mono-methylated metabolites (M10 and M11), three mono-methyl-glucuronidated metabolites (M19, M20, and M21), and one glucuronidated metabolite (M18) of 1,5-DCQA were exclusively detected in vitro, and that another two mono-methylates (M8 and M9) were only detected in the in vitro incubated samples and plasma, suggested that these mono-methylated, mono-methyl-glucuronidated, and glucuronidated metabolites were the primary metabolites of 1,5-DCQA. Given sufficient reaction time and enzymes, they would act as substrates and undergo further methylation and/or glucuronidation to form the di-methylated and di-methyl-glucuronidated metabolites. Therefore, it was not surprising that there were few intermediate metabolites but many di-methylated and di-methyl-glucuronidated metabolites detected in rats. This finding suggests that the biotransformation of 1,5-DCQA in vivo is considerably rapid and efficient and that 1,5-DCQA may be metabolized by two pathways, namely, methylation followed by glucuronidation, and glucuronidation followed by methylation.

Based on the results obtained in the in vitro studies, we tried to speculate about the structures of the methylated metabolites. In the structure of 1,5-DCQA, there are four phenolic hydroxyl groups (3′,4′- and 3′,4′-ortho-dihydroxy), which provide four sites for the methylation catalyzed by COMT. M8, M9, M10, and M11 have been identified as four such mono-methylated metabolites, in which the O-methylation positions of M8 and M9 have been confirmed to be on the two meta-hydroxyl groups of 1,5-DCQA. Accordingly, the O-methylation positions of M10 and M11 are proposed to be on the two

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**TABLE 1**

Summary of key LC/MS data of the metabolites found in vivo and in vitro

For chromatographic and spectroscopic conditions, see Materials and Methods.

<table>
<thead>
<tr>
<th>Compound</th>
<th>t_R (min)</th>
<th>MS [M – H]^− (m/z)</th>
<th>MS/MS (Fragment Ions)</th>
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<tr>
<td>1,5-DCQA</td>
<td>14.1</td>
<td>515</td>
<td>353 (100), 191 (61), 179 (7), 335 (5)</td>
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<tr>
<td>M1</td>
<td>10.6</td>
<td>719</td>
<td>543 (100), 367 (41)</td>
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<td>M2</td>
<td>11.9</td>
<td>705</td>
<td>529 (100), 353 (3)</td>
</tr>
<tr>
<td>M3</td>
<td>13.5</td>
<td>543</td>
<td>367 (100), 349 (12), 193 (3)</td>
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<td>13.8</td>
<td>719</td>
<td>543 (100)</td>
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<tr>
<td>M5</td>
<td>14.8</td>
<td>719</td>
<td>543 (100), 525(80)</td>
</tr>
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<td>543</td>
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<td>M7</td>
<td>18.2</td>
<td>719</td>
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</tr>
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<td>543</td>
<td>349 (100), 367(3)</td>
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**Fig. 3.** DAD profiles of incubation of 1,5-DCQA with rat intestinal cytosol and microsomes in the absence of 3,5-dinitrocatechol (A) and in the presence of 3,5-dinitrocatechol (B).
para-hydroxyl groups. As for the di-methylated products, M12, M13, and M14, because the two methylation positions were on the two respective caffeoyl groups of 1,5-DCQA, they were proposed to be 3′,3‴-, 3′,4‴-, 3″,4‴-, or 4‴,4‴-di-methylated metabolites. Zhu et al. (2000) reported that COMT at pH 7.4 predominantly methylated the meta-hydroxyl groups of flavonoids, such as norepinephrine and quercetin. If this were the same case for 1,5-DCQA, the occurrence of the methylation on 4‴- and 4‴-phenolic hydroxyls simultaneously would be more difficult. Since M12 has been confirmed as 3′,3‴-di-methyl 1,5-DCQA, M13 and M14 are identified as 3″,4‴- and/or 3‴,4‴-di-methyl 1,5-DCQA, respectively.

It was very interesting to notice that in addition to M12–M14, there were still six more metabolites (M3, M6, M22, M15, M16, and M17) identified as di-methylated metabolites of 1,5-DCQA. According to their retention properties on the reversed-phase column, we tentatively assigned M3, M6, and M22 as group I, M12–M14 as group II, and M15–M17 as group III. Slanina et al. (2001) reported that 1,3-DCQA (1,3-dicaffeoylquinic acid) could be formed from 1,5-DCQA by intramolecular transesterification. We supposed that 1,5-DCQA might undergo the reaction in a similar pattern in rats. If that were the case, the isomerization among the three groups of the di-methylated metabolites should be due to the intramolecular transesterification, for instance, isomerization of 1,5-DCQA to 3,5-DCQA, and the isomerization within each group should arise from the different methylation sites on the four phenolic hydroxyls, just like M12–M14 in group II. However, stereochemical trans-configuration of the quinic acid moiety could not be ruled out.

There were many glucuronidated metabolites formed in vivo and in vitro, which possessed increased aqueous solubility in comparison with their substrates. A portion of these glucuronidated metabolites excreted in bile might undergo enterohepatic circulation via glucuronidase-catalyzed hydrolysis. As for their glucuronidation site, if the four mono-methyl-glucuroconjugates (M2 and M19–M21) corresponded to the four mono-methylated metabolites (M8–M11) of 1,5-DCQA, it seemed that the methylation and glucuronidation would not affect each other, and the glucuronidation position might be on the carboxyl group or one of the three hydroxyl groups of the quinic acid moiety of 1,5-DCQA. Based on the information obtained in this study, a plausible scheme for the metabolic pathways of 1,5-DCQA in rats is shown in Fig. 4, not including the intramolecular transesterification. Two di-methyl-glucuroconjugates (M1 and M7) were also not included in Fig. 4, because it was uncertain whether they were the glucuroconjugates of the di-methylated metabolites in groups I and III.

Many dietary polyphenols with structures similar to that of 1,5-DCQA, e.g., chlorogenic acid, can be extensively degraded by the microflora of rat and/or human into low molecular weight compounds, which are then absorbed by the gut (Choudhury et al., 1999; Olthof et al., 2001, 2003; Gonthier et al., 2003). In the present study, some low molecular weight compounds with quasi-molecular ions ([M – H]⁻)
at mlz 178, 193, and 191 were observed in rat urine, but because they were also detected in blank samples, we could not draw any conclusion from the results. Further work will be done to address the role of microflora in the metabolism of 1,5-DCQA. In addition, trace amounts of sulfation products with deprotonated molecular ions at mlz 595, 623, and 609 were detected in bile and urine samples; owing to their low levels, the results require further investigation.

In conclusion, this paper reports for the first time that 1,5-DCQA can be efficiently O-methylated and glucurononidated by COMT and UGTs in rat small intestine and liver with the formation of a large number of metabolites in vivo and in vitro. The results suggest that metabolism is one factor that can reduce the amount of 1,5-DCQA in circulation, whereas the formation of bioactive metabolites may make an important contribution to the anti-HIV effect of 1,5-DCQA. The information gained from our study could be very useful for the further metabolic studies of other DCQAs.

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